Sinapoylglucose: Malate Sinapoyltransferase Activity in Arabidopsis thaliana and Brassica rapa

Hans-Peter Mock, Thomas Vogt, and Dieter Strack

Institut für Pharmazeutische Biologie der Technischen Universität Braunschweig,
Mendelssohnstraße 1, D-W-3300 Braunschweig, Bundesrepublik Deutschland

Z. Naturforsch. 47c, 680–682 (1992); received August 19, 1992

Arabidopsis thaliana, Brassica rapa, Acyltransferase, Glucose Ester, Hydroxycinnamic Acid Ester

Protein preparations from Arabidopsis thaliana and Brassica rapa catalyzed the formation of O-sinapoyl-L-malate using O-sinapoyl-β-glucose as acyl donor. The enzyme involved, 1-O-sinapoyl-β-glucose:1-malate O-sinapoyltransferase (SMT; EC 2.3.1.1–), catalyzes in the cotyledons of the seedlings the key step in the conversion of the seed constituent sinapine (O-sinapoylcholine) to sinapylmalate by way of the intermediate sinapoylglycerol.

Introduction

Most species of the Brassicaceae accumulate the seed constituent sinapine (O-sinapoylcholine) [1, 2] whose formation is catalyzed by a 1-O-sinapoyl-β-glucose:choline O-sinapoyltransferase (sinapine synthase; EC 2.3.1.1–) [3]. During seed germination and seedling development, sinapine is hydrolyzed by a specific sinapine esterase in Raphanus sativus (EC 3.1.1.49) [4, 5]. The liberated choline is used for the biosynthesis of phosphatidylcholine [6], and sinapic acid is conjugated to the energy-rich 1-O-sinapoyl-β-glucose (SinGlc) [7], catalyzed by an UDP-glucose:sinapic acid O-glucosyltransferase (EC 2.4.1.120) [8, 9]. It has been shown so far for R. sativus [10] and Brassica napus [11] that SinGlc serves as the acyl donor in the formation of O-sinapoylmalate (SinMal), catalyzed by a 1-O-sinapoyl-β-glucose:1-malate O-sinapoyltransferase (SMT; EC 2.3.1.1–), which has recently been purified from R. sativus seedlings [12]. In this paper we show the presence of SMT also in Arabidopsis thaliana and Brassica rapa, and from changes in metabolic concentrations the conversion of sinapine to SinMal via SinGlc as the intermediate is suggested.

Materials and Methods

Plant material and growing conditions

Seeds of Arabidopsis thaliana L. (seed line Aa−O) were provided by Prof. Dr. A. R. Kranz (Botanisches Institut der Universität, Frankfurt, F.R.G.). Seeds of Brassica rapa L. ssp. oleifera (DC.) METZG. (B. campestris L.) were purchased from Schmitz & Laux, Hilden, F.R.G. Seedlings and young plants were grown in the greenhouse. Plant material harvested was immediately frozen in liquid nitrogen and stored at −80 °C.

Extraction and quantification of sinapic acid esters

Seeds (50 to 100), frozen seedlings, pairs of cotyledons or young plants (20 to 50) were ground in a mortar in the presence of liquid nitrogen. Aqueous methanol (80%, v/v) was added and the homogenate stirred for 30 min. After centrifugation (5 min, 16,000 × g) the supernatant was stored at −80 °C. Prior to HPLC for separation and quantification of the sinapic acid esters, extract aliquots were diluted with water to give 50% aq. methanol and centrifuged.

Extraction and estimation of SMT activity

Seeds (100), frozen seedlings, pairs of cotyledons or young plants (50) were ground in a mortar in the presence of liquid nitrogen. Polyclar AT (100 mg) and potassium phosphate buffer (4 °C; 100 mm; pH 6.0) were added, the homogenate stirred for 1 h at 4 °C, and then centrifuged (15 min, 20,000 × g). The supernatant was immediately assayed for SMT activity (B. rapa) or concentrated by ultrafiltration prior to activity determination (A. thaliana). The SMT activities were determined through quantification of the product SinMal by means of HPLC analysis of the supernatants from centrifuged SMT assays. The standard enzyme assays were carried out as described by

Reprint requests to D. Strack.

Verlag der Zeitschrift für Naturforschung, D-W-7400 Tübingen
0939–5075/92/0900–0680 $ 01.30/0

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung“) beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

High performance liquid chromatography (HPLC)

The liquid chromatograph and the chromatographic conditions as well as the quantitative standardization is described elsewhere [11].

Results and Discussion

This paper shows the presence of SMT in *Arabidopsis thaliana* and *Brassica rapa*. Table I lists the enzyme activities and the amounts of the product SinMal together with potential precursors sinapine and SinGlc in seeds and in developing young plants. Based on the same biochemical mechanisms found in *R. sativus* [14] and *B. napus* [11], there is a rapid degradation of sinapine during seed germination, followed by the SMT-catalyzed SinMal formation from the transiently accumulating intermediate SinGlc:

\[
\text{sinapine} \overset{(1)}{\rightarrow} \text{sinapic acid} \overset{(2)}{\rightarrow} \text{SinGlc} \overset{(3)}{\rightarrow} \text{SinMal}
\]

(1), sinapine esterase; (2), UDP-glucose: sinapic acid O-glucosyltransferase; (3), SMT.

In both plants, the time course of expression of SMT activity correlates well with the accumulation of SinMal. In *B. rapa* cotyledons the amount of SinMal corresponds to the amount of the seed constituent sinapine as the precursor, indicating interconversion reactions within a preformed sinapic acid pool. In young plants of *A. thaliana*, the amount of sinapine contributes to only a small portion (less than 1%) of the amounts accumulating SinMal. This discrepancy is due the fact that not only the seedlings but also leaves of the young plants such as the rosette leaves of *A. thaliana* (completely developed at day 24) were extracted. In the latter, sinapic acid for SinMal formation is obviously provided by de novo synthesis. Here the acyl donor SinGlc is detectable in trace amounts only and must underlie a high turnover rate. This is also possibly true for other members of the Brassicaceae shown so far to develop SMT activity, which may catalyze, along with SinMal, the formation of other malic acid esters of hydroxycinnamic acids, such as 4-coumaroyl-, caffeoyl- and feruloylmalate found in adult *R. sativus* plants [15–17]. Thus the PAL-independent interconversions of the sinapic acid esters [18] shown above, is restricted to the cotyledons. This was also found with *A. thaliana* (not documented) where the amount of SinMal accumulating in the cotyledons corresponds to the amount of sinapine hydrolyzed during seed germination.

The results from *B. rapa* touch another interesting point concerning plant genetics. In previous studies it was found that *B. oleracea* does not accumulate SinMal [18] nor could SMT activity be detected [H.-P. Mock and D. Strack, unpublished], whereas *B. napus* showed both SinMal accumulation and development of SMT activity [11]. *B. napus* is known to be an allopolyploid species containing the genomes of *B. rapa* and *B. oleracea*. Therefore it is reasonable to assume that the genome of *B. rapa* contributes to the expression of SMT activity in *B. napus*.

Further studies of the sinapic acid ester metabolism in members of the Brassicaceae focusing on expression and control of SMT activity [19] may help to understand the regulation of phenylpropanoid metabolism in plants. In addition it seems worthwhile to study further the distribution of SMT activity among members of the Brassicaceae.
Acknowledgements

Financial support to D. S. by the Deutsche Forschungsgemeinschaft (Bonn) and the Fonds der Chemischen Industrie (Frankfurt) is gratefully acknowledged. Some of the experiments with *A. thaliana* were carried by T. V. in the laboratory of B. E. Ellis (Department of Plant Science, University of British Columbia, Vancouver, Canada).